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Mycorrhizal fungal establishment in agricultural soils: factors determining inoculation success

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Summary

Soil biota provide a number of key ecological services to natural and agricultural ecosystems. Increasingly, inoculation of soils with beneficial soil biota is being considered as a tool to enhance plant productivity and sustainability of agricultural ecosystems. However, one important bottleneck is the establishment of viable microbial populations that can persist over multiple seasons.

Here, we explore the factors responsible for establishment of the beneficial soil fungi, arbuscular mycorrhizal fungi (AMF) that can enhance yield of a wide range of agricultural crops. We evaluate field application potential and discuss ecological and evolutionary factors responsible for application success.

We identified three factors that determine inoculation success and AM fungal persistence in soils: 1) species compatibility (can the introduced species thrive under the imposed circumstances?); 2) field carrying capacity (the habitat niche available to AMF); and 3) priority effects (the influence of timing and competition on the establishment of alternative stable communities).

We explore how these factors can be employed for establishment and persistence of AM fungi. We address the importance of inoculum choice, plant choice, management practices and timing of inoculation on the successful manipulation of the resulting AMF community.

Key words: arbuscular mycorrhizal fungi, community assembly, establishment, field inoculation, priority effects.

Introduction

One of the greatest challenges of the 21st century is to feed an increasing world population without exacerbating current environmental problems (Fitter, 2012). One promising approach is to increase the utilization efficiency of scarce non-renewable fertilizers. This has the potential to simultaneously increase plant productivity, while reducing pressures on the environment. Soil microbes offer largely unexplored potential to increase agricultural yields and productivity in a low-input manner.

Evolutionary and ecological research is unveiling the various mechanisms by which soil microbes can stimulate plant productivity (Van der Heijden *et al.*, 2008). In particular, rhizosphere symbionts named arbuscular mycorrhizal fungi (AMF), have received considerable attention as a potential low-input solution to increasing nutrient uptake efficiency of crop hosts. The majority of plant species, including most agricultural crops, enter into a symbiosis with mycorrhizal fungi, exchanging plant sugars for fungal derived nutrients, such as phosphorus and nitrogen. Apart from nutritional benefits, they are also known to increase soil structure and suppress diseases.

Given the potential benefits to agricultural productivity (see: Lekberg & Koide (2005) for review), it is not surprising that manipulation of AMF communities (either by inoculation with particular strains or through management of resident communities) has been attempted at the field scale. The implicit assumption of inoculation is that these systems are limited in either abundance or functioning of AMF, and that this limitation can be reversed through application of fungal inoculum. However, limitation may not only arise from insufficient availability or quality of AM fungal propagules, but also from complex ecological and

evolutionary dynamics of plant-fungal interactions. Here we synthesize the current knowledge on AMF establishment and loss, analyzing the success and failure of field inoculations using an ecological and evolutionary vantage point. Our ultimate goal is to predict how this symbiosis may be best optimized at the field level to increase plant productivity.

Are agricultural fields limited by AMF?

An important starting point is to determine whether AMF are limiting to processes in agricultural settings. Limitation has the potential to occur in at least two different forms: abundance and diversity. Abundance of AMF has been found to be negatively associated with intensive agricultural production (Smith & Read, 2008). Tillage, high levels of nutrients (particularly phosphorus) and frequent fallow-periods are all predicted to decrease the absolute abundance of viable AMF propagules, such as spores and infective mycelium (Karasawa & Takebe, 2011; Schnoor *et al.*, 2011). If sufficient root colonization is not achieved, suboptimal plant growth can occur. Under such circumstances, inoculation offers a potential solution to increase colonization and benefits to crop hosts.

The other form of limitation is genetic, meaning levels of AMF diversity. Agricultural systems typically harbour lower AMF diversity than natural systems (Verbruggen *et al.*, 2010), and these systems are often dominated by a few select taxa within the AMF order *Glomerales* (Oehl *et al.*, 2010). For example, one particular genotype of *Funneliformis mosseae* was found to have a global distribution, potentially related to its spread and successful adaptation to agricultural conditions (Rosendahl *et al.*, 2009). A reduction of diversity can limit AMF-derived benefits if: (i) different AMF species or genotypes provide complementary benefits, or (ii) the potentially highest yielding plant-fungal combination is not formed. Both of these mechanisms are known to occur when AMF diversity is

manipulated (Wagg *et al.*, 2011). Adding inoculum has the theoretical potential to alleviate these types of limitations.

In the case of abundance, there is strong evidence that AMF colonization and spore number can be stimulated by altering management practices, for example mediating fertilization regime (Smith & Read, 2008), reducing fallow and/or growth of non-mycorrhizal crops (Karasawa & Takebe, 2011) and introducing organic management schemes, including the planting of temporary grass-clover pastures (Verbruggen *et al.*, 2012b). Loss of the AM fungal gene-pool may be harder to reverse, as it requires spread and establishment (i.e. dispersal) into the system. In a recent meta-analysis of 111 sites (which included a variety of habitats), AMF dispersal was estimated to be very low; a large majority of taxa was only found at single sites (Kivlin *et al.*, 2011). Limited movement (due to underground spore-formation) and strong local adaptation (Johnson *et al.*, 2010) will potentially amplify each other, and may strongly hamper invasibility of local AMF assemblages by non-local strains.

Because the type of limitation (genetic *versus* abundance) may have important implications for the merits of AMF application, it is important to distinguish them. Experiments in which AMF are applied to agricultural fields usually manipulate both abundance and species composition of AMF, making it difficult to disentangle their independent effects. However, in a thorough meta-analysis of various trials, effect of inoculation was explicitly compared with changes in indigenous AMF abundance via specific management changes (Lekberg & Koide, 2005). The results demonstrated that on average, inoculation increased root colonization by 29%, resulting in a significant plant biomass increase of 23%. This suggests that the introduced strain performed a non-redundant function, resulting in increased plant nutrition. However, these effects were similar in trials where AMF abundance was increased by

alternative management, particularly by shortening of the fallow period. Thus, plant responses to inoculation may to a large extent be driven by increases in AMF abundance, rather than the introduction of new strains. This is in line with recent work suggesting that native and exotic inocula can be similarly effective in increasing plant growth (Pellegrino *et al.*, 2011).

Collectively, this evidence supports the idea that inoculation effects may often be transient, and related to abundance, rather than identity effects. Nonetheless, if an agricultural field is, in principle, conducive to a better functioning AMF community but is limited by dispersal, inoculation may have long-term beneficial effects. This would require successful establishment on a crop and - for long-term benefits (e.g. throughout a crop rotation sequence) - persistence of an introduced AM fungal strain. In the next section we will explore which processes determine whether AMF will establish.

Which factors contribute to establishment?

Compatibility

How compatible are introduced species with local agricultural conditions? Agricultural fields are subject to intense selection pressures from management practices (Rosendahl *et al.*, 2009). As a result, they are often occupied by fewer taxa than more natural systems, and these taxa are presumably well-adapted to agricultural conditions (Oehl *et al.*, 2010; Schnoor *et al.*, 2011). Establishment of new taxa may be difficult if they are to compete with well-adapted local communities. One promising approach would be to match potential inoculants with specific field conditions, such as tillage environment (Schnoor *et al.*, 2011), soil type and pH (Oehl *et al.*, 2010) and breadth of potential hosts (Öpik & Moora, 2012). Particularly this last point deserves attention, because it is becoming more and more apparent that some AMF taxa

can be host- or habitat “specialists”, while others are “generalists” (Öpik & Moora, 2012). Since crops are usually rotated, and weeds can serve as additional inoculum sources, an AMF strain that is a plant-host generalist - all else being equal - is likely to have an enhanced establishment and persistence.

Sites that have a low AMF species diversity due to historical mismanagement, are expected to particularly benefit from inoculation, as these are the cases in which AMF limitations are most likely to occur (Wagg *et al.*, 2011). Moreover, these sites may also exhibit an increased compatibility with inoculated species, provided that the low diversity means there are more unoccupied niches available. If the low complexity of agricultural systems is responsible for relatively low species diversity (i.e. dominance is no longer countered by non-equilibrium dynamics typical of more complex ecosystems), this may actually favour - instead of limit - establishment of particularly suitable strains. In order to still maintain a diverse AMF community, circumstances (e.g. management-regimes) responsible for the low diversity have to be modified to a certain extent.

Carrying capacity and abundance

Two aspects of AMF abundance are strongly interrelated, and are expected to strongly affect establishment: (i) inoculum quantity and (ii) the extent to which the particular system supports AM fungal populations. Some agricultural soils may have a reduced “carrying capacity”, caused by low plant allocation towards AMF. This is thought to occur as a response to culturing of non-host plants, high nutrient levels, or other adverse environmental factors. For instance, high phosphorus levels do not typically favour a large AMF community because these conditions often suppress root colonization and thus growth of AMF (Fig. 1) (Smith & Read, 2008; Verbruggen *et al.*, 2012b). For introduced AMF, this may be a

problem: if AMF suffer from reduced fitness at low population sizes, they will be easily outcompeted by native AM fungi. Mechanisms through which this can occur include demographic stochasticity or “Allee effects”, where abundant AMF experience a higher fitness. The latter phenomenon is common across a wide diversity of organisms, and may operate in AMF if abundant strains have a strong tendency to engage in anastomosis; i.e. hyphal fusion resulting in direct physiological links between mycelia. At higher density AMF will be better able to reach conspecific strains. This will increase the volume of soil and plant roots colonized, more than proportionally (Rosendahl, 2008). However even in absence of anastomosis, there may be an abundance threshold below which taxa are disadvantaged. Relatively abundant taxa may be favoured through a positive feedback. For example, if intact mycelia are present early in the season, abundant taxa can simultaneously colonize multiple plants leading to strong dominance of such taxa in AM fungal assemblages (Dumbrell *et al.*, 2011).

There are only few studies that have assessed AMF establishment multiple years after inoculation. This means that information on the critical propagule abundance that ensures successful establishment is currently lacking. In recent years, the possibility of tracking inoculum strains over multiple years has greatly increased through the development of molecular methods (see Table 1). For instance, in one multi-year tracking study, there was strong evidence of persistence of at least one of two *Funneliformis mosseae* isolates (Pellegrino *et al.*, 2012). However, levels of inoculum applied were roughly equivalent to native population densities (Pellegrino *et al.*, 2012). If such doses are a requirement for successful establishment and persistence of AM fungi, this seriously reduces the feasibility of inoculation approaches: the amount applied in this study would scale up application rates of 7 ton per hectare of soil (Table 1).

Priority effects

One phenomenon that can strongly affect community composition is priority effects; i.e. the process by which initial species present in a community determine the eventual community composition. This process can amplify or negate any inherent competitive superiority. For instance, in fungi involved in wood decay, order of arrival has a critical effect on eventual community composition (Dickie *et al.*, 2012). These priority effects even outweighed the effect of fertilization, which is considered among the strongest determinants of species sorting in these fungi (Dickie *et al.*, 2012). In AMF, Lekberg *et al.* (2012) measured whether physical disturbance affects communities in a predictable way, testing whether disturbance benefits some AM fungal taxa over others. While disturbance was not found to directly predict species composition, they proposed that priority effects were responsible for community differences after disturbance. If eventual community composition is contingent on small differences in initial conditions, they may be influenced relatively easily. Such stochasticity has been argued to be a major determining factor in several studies on AM fungal communities (e.g. Dumbrell *et al.*, 2011; Caruso *et al.*, 2012).

Estimates of the strength of priority effects on AM fungal communities are limited, but indirect evidence suggests they can be important: Hausmann & Hawkes (2010) found that the order in which host plants (colonized by different AMF) established was a decisive factor in determining the resulting taxa of the community. Likewise, when plants already colonized by a particular AMF were introduced to a natural AMF community, the eventual diversity and composition of the resulting community was largely altered compared to uninoculated plants (Mummey *et al.*, 2009). One important question is whether effects of priority are symmetric: if plant colonization by one taxon has a prohibitive or stimulatory effect on a subsequent

colonizer, does the same apply if the order is reversed? If this is the case, the effect on community dynamics might be relatively straightforward to predict. If however there is less regularity in priority effects, these effects will be very complex and community-specific, limiting the possibility to predict establishment chances without extensive prior knowledge of the system.

How can these processes affect inoculation success?

Species compatibility, carrying capacity, and priority effects are all processes that directly or indirectly affect competition for root or soil space, and therefore are important in determining persistence and success of specific fungal taxa. If we understand how these processes operate in plant-AMF interactions, we can optimize management to stimulate the establishment of introduced strains (for a schematic overview of these processes see Fig. 2).

One potentially successful approach may be to find which AMF families or functional types are missing from particular habitats and add agriculturally ‘compatible’ representatives. Such inoculation attempts may have an increased establishment success because of “unoccupied niches”. For example in one study, the AMF species *Claroideoglossum etunicatum* was particularly successful (Table 1) (Farmer *et al.*, 2007). Of the three inoculants assessed, this species was the only one for which the field lacked any other representatives of its particular family, which is likely to have contributed to its success. When there is no prior knowledge about what niches remain unoccupied - which will often be the case - one could consider applying a mixture of species which will increase the likelihood that at least one species is able to establish and persist. However, since this will simultaneously decrease the absolute amount of this species in the inoculum, species inclusion should be carefully considered.

In order to influence carrying capacity, crop choice and fertilization may be particularly important. There is a strong connection between soil P levels and root colonization, where root colonization decreases strongly with increasing P (Fig. 1). Decreasing root colonization corresponds with active downregulation of mycorrhization by plants, and thus carbon allocation, when plants are less dependent on AMF for P supply (e.g. Lekberg & Koide, 2005; Smith & Read, 2008). Moreover, there is high variation among plant species in their reliance on AMF (Smith & Read, 2008). Thus, choosing a highly mycorrhizal - dependent crop and optimizing the nutrient environment are two management factors that are very likely to increase the establishment success of new strains.

To take advantage of priority effects, timing of inoculation may be crucial, although already in most trials the inoculum is added directly to the soil during plant germination (e.g. Pellegrino *et al.*, 2011). In some instances, seedlings could be inoculated prior to planting (Farmer *et al.*, 2007; Mummey *et al.*, 2009), potentially acting as reservoirs to facilitate spread of new material. Likewise, particular strains could be given a head-start, by applying inoculum in a very patchy manner in the field instead of distributing it evenly. When distributed in this manner, high-density areas would experience reduced inter-specific competition, potentially increasing chances of establishment.

Tillage is a management practice that could be optimized to increase successful establishment of AMF. Tillage greatly influences spatial aggregation, and thereby competitive interactions. In two recent studies where spatial distribution of two AMF species was varied (more - or less mixing of soil), strong differences in respective species proliferation were found (Bever *et al.*, 2009; Verbruggen *et al.*, 2012a). Future studies should test whether there is an 'optimum' spatial structure that increases the establishment success of 'rare' introduced AMF. This could

be determined under realistic conditions (i.e. at the field scale with different tillage levels) to assess the optimal level of soil mixing. Given the potential importance of spatial aggregation of both resident and introduced AMF, 'intensive' tillage prior to inoculation will reduce aggregation of the resident fungi, and strongly affect the success of the introduced new one. Mixing can increase the number of competitors present at any given place, thereby potentially increasing the chances the new fungus will be displaced (*sensu* Verbruggen *et al.*, 2012a). However, if introduced strains are applied patchily after tillage, it is possible that the new fungus will be locally much more abundant than others because the latter are 'diluted' through mixing. This may enhance the competitive edge of the new fungus, through abundance and priority effects. These are only two of many potential outcomes, all of which deserve to be tested in AMF application trials.

Perspectives

Here we have reviewed emerging scientific work on processes that influence the success of introduced AMF strains. Using this information, we have highlighted new approaches that hold potential to enhance chances of successful inoculation and warrant further investigation. Research efforts should also be directed towards negative (side-) effects of introducing AMF: concerns about introducing non-native microbes have been raised, and caution should be taken in regards to distributing inocula over large geographical areas (Schwartz *et al.*, 2006). Another potential concern is the risk that introduction of AMF will cause 'outbreeding depression' through genetic exchange of resident and introduced strains. Colard *et al.* (2011) found genetic exchange between AMF can both increase and decrease mutualistic quality. Consequences of genetic exchange between native and alien strains on mutualistic quality remain a research priority.

Another important avenue of research is that of indirect effects. Sometimes positive results of inoculation occur that are not related to root colonization by the introduced AMF (e.g. Alguacil *et al.*, 2011; Pellegrino *et al.*, 2012). These may be driven by indirect effects on plant-soil interactions. We must have a better understanding of how these indirect and direct effects of “new” species interact in order to fully profit from inoculation, while minimizing negative side-effects. It is important to consider that effects of AMF on plants range from antagonistic to mutualistic and that several plants, including several agricultural weeds and some crops can be suppressed by AMF when conditions are unfavourable. Moreover, in order to make commercial application of AMF feasible, a general framework is required which predicts under which conditions inoculation will contribute to enhanced yield and agricultural sustainability in an explicit economical context.

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